Landes Bioscience www.landesbioscience.com

Supplemental Material to:

Zili Zhai, Feng Wu, Fengshi Dong, Alice Y Chuang, Jeannette S Messer, David L Boone, and John H Kwon

Human autophagy gene *ATG16L1* is post-transcriptionally regulated by *MIR142-3p*

Autophagy 2014; 10(3)
http://dx.doi.org/10.4161/auto.27553

www.landesbioscience.com/journals/autophagy/article/27553

Human autophagy gene *ATG16L1* is post-transcriptionally regulated by *MIR142-3p*

Zili Zhai, Feng Wu, Fengshi Dong, Alice Y. Chuang, Jeannette S. Messer, David L. Boone, and John H. Kwon*

Department of Medicine, Section of Gastroenterology, The University of Chicago, Chicago, IL 60637, USA

*Correspondence to: Department of Medicine, Section of Gastroenterology, The University of Chicago, Chicago, IL 60637, USA. Tel.: 773.702.5935; Fax: 773.702.2281; Email: jkwon@medicine.bsd.uchicago.edu

Supplementary data:

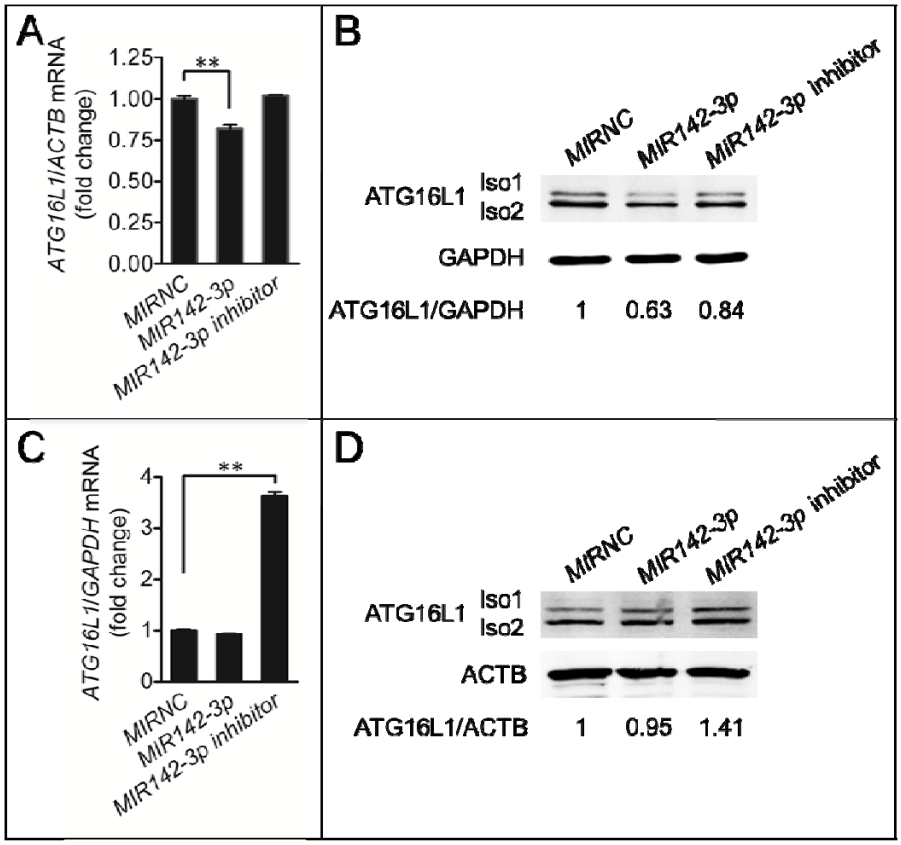

Figure S1. The transfection effects of the *MIR142-3p* mimic and inhibitor on *ATG16L1* expression. HCT116 cells were transfected with 100 nM of the *MIR142-3p* mimic, inhibitor or *MIRNC*. At 24 h post-transfection, (**A**) real time RT-PCR analysis of *ATG16L1* mRNA expression with *ACTB* as an internal standard. The data are expressed as the mean ± SEM (n=3). (**B**)

Western blot analysis of ATG16L1 protein expression. The density of ATG16L1 bands was quantitated using the Odyssey infrared imaging system and normalized to that of the corresponding loading control GAPDH with the same treatment. Representative bands are shown. Jurkat cells were transfected with 50 nM of the *MIR142-3p* mimic, inhibitor or *MIRNC*. At 24 to 48 h post-transfection, (**C**) real time RT-PCR analysis of *ATG16L1* mRNA expression with *GAPDH* as an internal standard. The data are expressed as the mean ± SEM (n=3). (**D**) Western blot analysis of ATG16L1 protein expression. The density of ATG16L1 bands was quantitated and normalized to that of the corresponding loading control ACTB with the same treatment. Representative bands are shown. ***P*<0.01 vs *MIRNC*.

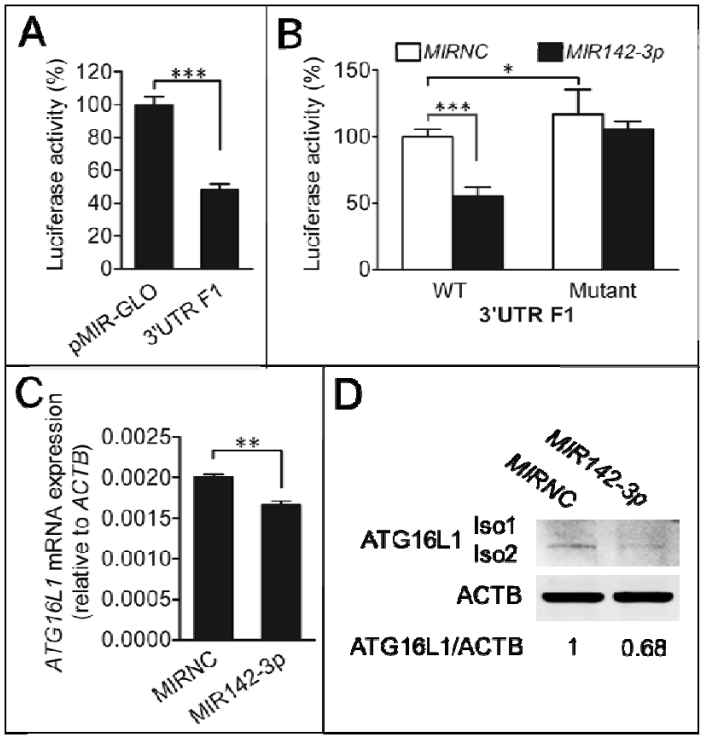

Figure S2. *MIR142-3p* targets *ATG16L1* in Caco-2 cells. Similar as HCT116 cells, Caco-2 cells were examined for luciferase activity and *ATG16L1* expression. (**A**) Cells were transfected with the WT *ATG16L1* 3'UTR F1 vector or pMIR-GLO vector control (200 ng/ml) and dual luciferase activities were determined 48 h later. The data are expressed as the mean ± SEM (n=4). ****P*<0.001 vs the control pMIR-GLO vector. (**B**) Cells were cotransfected with the WT or mutated *ATG16L1* F1 vector (200 ng/ml) and the *MIR142-3p* mimic or *MIRNC* (10 nM) and dual luciferase activities were determined 48 h later. The data are expressed as the mean ± SEM (n=5 or 6). **P*<0.05 and ****P*<0.001 vs the control pMIR-GLO vector. (**C**) Real time RT-PCR analysis of *ATG16L1* gene expression following transfection of *MIR142-3p* or *MIRNC* (50 nM) for 48 h. The data are expressed as the mean ± SEM (n=3). ***P*<0.01 vs the *MIRNC*. (**D**) Western blot analysis

of ATG16L1 expression 48 h post-transfection. The density of ATG16L1 bands was quantitated and normalized to ACTB.

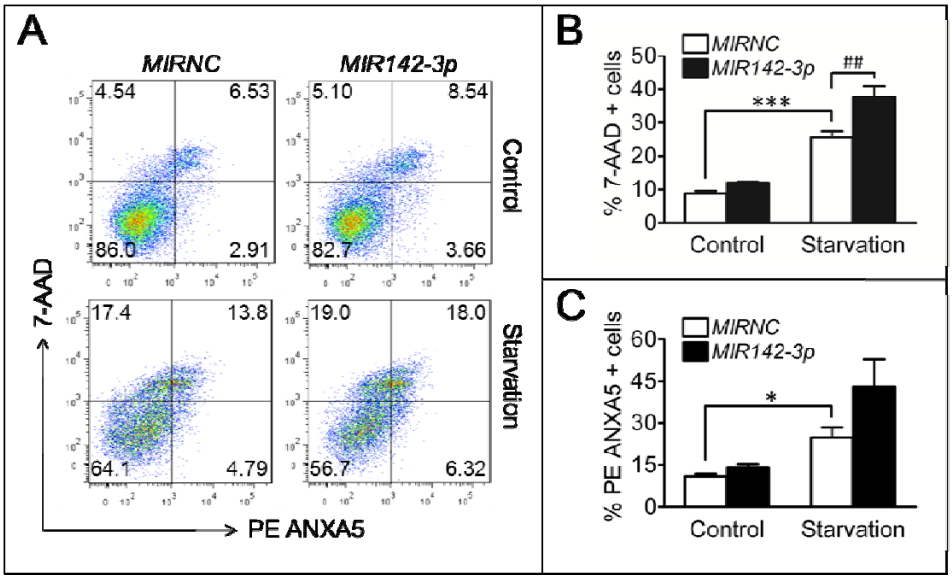

Figure S3. Flow cytometric analysis of cell death and apoptosis following *MIR142-3p* overexpression in HCT116 cells. Cells were seeded in 12-well plates. After transfection with the *MIR142-3p* mimic or *MIRNC* (50 nM), cells were incubated in complete medium or in EBSS for 18 to 22 h. Apoptosis and cell death were assessed by PE ANXA5/Annexin V and 7-AAD staining and flow cytometric analysis. For each sample, 20,000 events were collected and percent gated cell count was obtained. (**A**) Representative dot plots. (**B**) Cell death indicated by the increased percentage of 7-AAD positive cells. (**C**) Apoptosis indicated by the increased percentage of PE ANXA5-positive cells. The data are expressed as the mean ± SEM (n=4). **P*<0.05 and ****P*<0.001 vs the unstirred control transfected with *MIRNC*. $^{\#\#}$*P*<0.01 vs the starved control transfected with *MIRNC*.

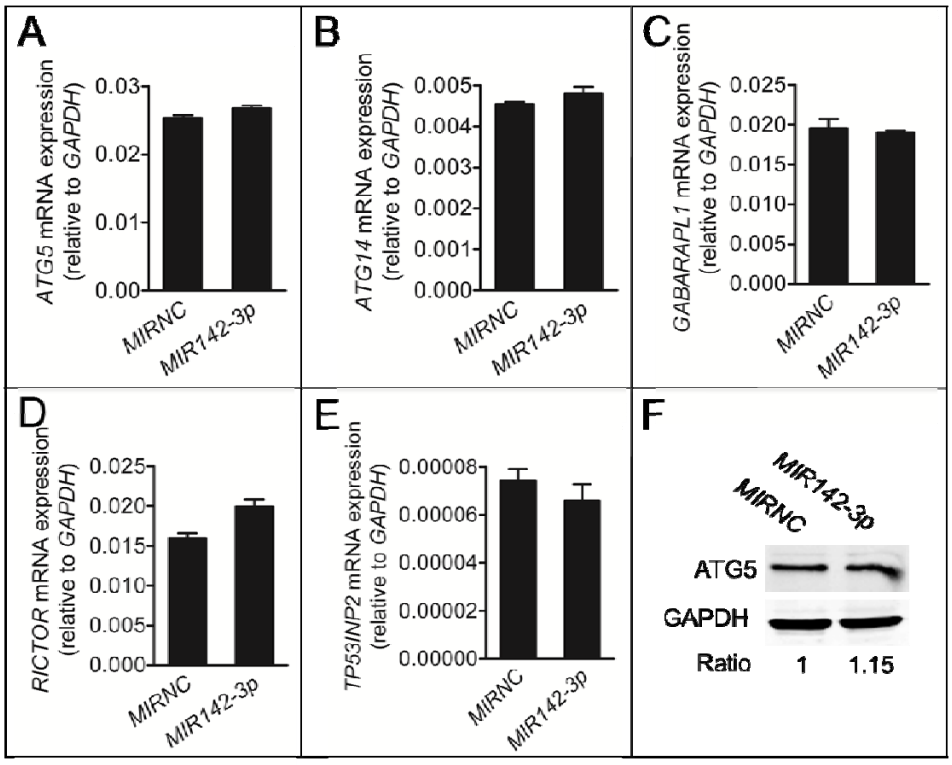

Figure S4. The effect of *MIR142-3p* mimic transfection on the expression levels of *ATG5*, *ATG14*, *GABARAPL1*, *RICTOR*, and *TP53INP2*. HCT116 cells were transfected with the *MIR142-3p* mimic or *MIRNC* (50 nM) for 24 or 48 h. (**A-E**) Total RNA was extracted and assayed for indicated gene mRNA expression by real time RT-PCR. The data are expressed as the mean ± SEM (n=3 for *ATG5* and *TP53INP*; n=6 for *ATG14*, *GABARAPL1*, *RICTOR*). (**F**) Western blot analysis of ATG5 protein expression. The density of ATG5 bands was quantitated and normalized to that of the corresponding loading control GAPDH with the same treatment. Representative bands are shown.